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Electrochemical detection of ractopamine at arrays of micro-liquid | liquid interfaces

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ABSTRACT

The behaviour of protonated ractopamine ($RacH^+$) at an array of micro-interfaces between two immiscible electrolyte solutions (micro-ITIES) was investigated via cyclic voltammetry (CV) and linear sweep stripping voltammetry (LSSV). The micro-ITIES array was formed at silicon membranes containing 30 pores of radius 11.09 ± 0.12 μm and pore centre-to-centre separation of 18.4 ± 2.1 times the pore radius. CV shows that $RacH^+$ transferred across the water | 1,6-dichlorohexane μITIES array at a very positive applied potential, close to the upper limit of the potential window. Nevertheless, CV was used in the estimation of some of the drug's thermodynamic parameters, such as the formal transfer potential and the Gibbs transfer energy. LSSV was implemented by pre-concentration of the drug, into the organic phase, followed by voltammetric detection, based on the back-transfer of $RacH^+$ from the organic to aqueous phase. Under optimised pre-concentration and detection conditions, a limit of detection of 0.1 μM was achieved. In addition, the impact of substances such as sugar, ascorbic acid, metal ions, amino acid and urea on $RacH^+$ detection was assessed. The detection of $RacH^+$ in artificial serum indicated that the presence of serum protein interferes in the detection signal, so that sample deproteinisation is required for feasible bioanalytical applications.

1. Introduction

Ion transfer across the interface between two immiscible electrolyte solutions (ITIES) [1–4] has received increasing interest in the field of electrochemistry, liquid | liquid extraction and membrane transport. Electrochemistry at liquid | liquid interfaces has moved from the transfer of model ions such as tetraalkylammonium ions to the detection of molecules of biological importance such as proteins, peptides, amino acids, drugs, neurotransmitters, food additives and DNA [5]. Thus it plays a potentially important role in new bioanalytical methods.

Ractopamine (Rac) (Fig. 1(a)) is a phenyl β-ethanolamine, with β-adrenergic agonist properties [6–9]. It is primarily used as a therapeutic drug for treatment of pulmonary diseases such as asthma in human and veterinary medicine [6,7]. Unfortunately, this substance is also illegally applied in the livestock industry as a nutrient repartitioning agent. Research shows that β-agonists divert fat deposition to the production of muscle tissues by increasing nitrogen retention, protein synthesis and lipolysis [6,7,9–11]. It also improves growth rate and feed conversion when fed to livestock (such as calves, poultry etc.) [8,9,12]. Recently, veterinary drug residues have become a public food safety concern where ractopamine-treated animals may pose adverse effects on human health, especially in the cardiovascular and central nervous systems [6,7,9,12,13]. Thus, it is banned in many countries, including within the European Union and China, although it is approved by the United States' Food and Drug Administration (U.S. FDA) [12,14–16]. As a result, rapid, simple and sensitive analytical methods for the detection of ractopamine residues are required.

To date, various analytical methods have been reported for the detection of ractopamine, such as immunoassays [9,10,17], electrochemical methods [6,7,13,16], gas chromatography–mass spectrometry [11], liquid chromatography tandem mass spectrometry [12,18] and high performance liquid chromatography [8,14,15]. Electrochemical methods offer the advantages of low instrumental cost and fast analysis, and thus may be the preferred methods in ractopamine detection [7]. Despite the fact that many electrochemical methods have been developed, those studies focused on solid | liquid interfaces, using primarily cyclic voltammetry (CV) [6,7,16]. The two phenolic groups in ractopamine are easily oxidised [6,7,13]. Differential pulse voltammetry (DPV) has also been employed for the detection of ractopamine [6,7,13].

To the best of our knowledge, no studies have been reported on the electrochemical detection of ionised ractopamine based on transfer across the ITIES. Thus, this study opens up the possibilities

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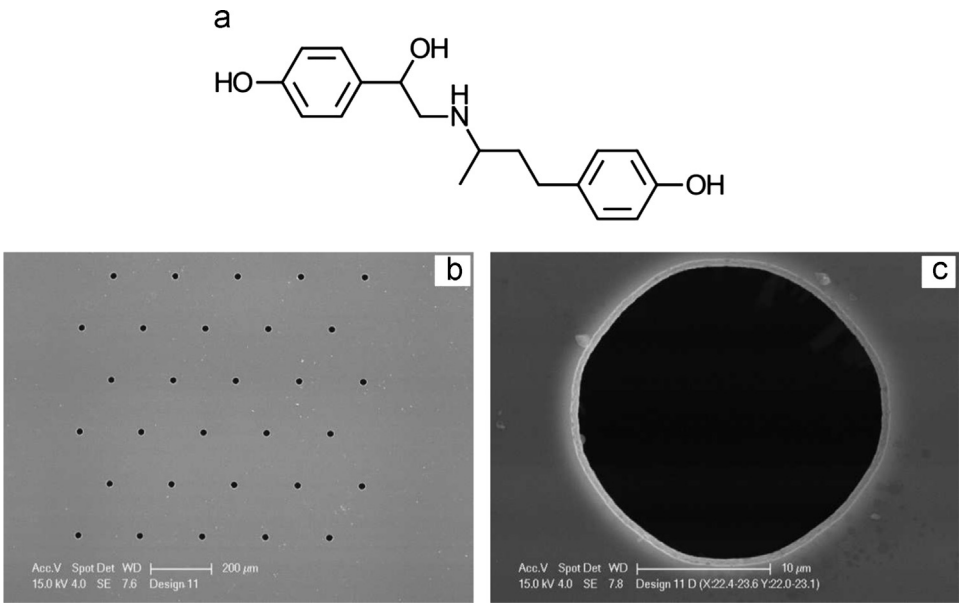

Fig. 1. (a) Molecular structure of ractopamine (b) SEM image of the full micropore array silicon membrane, and (c) SEM of a single micropore within the array.

for the detection of ractopamine based on charge transfer across micro-ITIES. However, the detection of other drugs at the ITIES via various electrochemical methods has been reported in the literature, namely the anticancer drug daunorubicin at a microporous polyethylene terephthalate (PET) membrane-supported ITIES [19], catamphiphilic drugs at a solvent polymeric membrane [20], and β-blocker drugs (propranolol, timolol and sotalol) at a microporous silicon membrane-supported ITIES [21]. Besides analytical studies, the ability of the ITIES to mimic the drug transfer across biological membranes has offered insight into mechanisms of drug action [19]. Voltammetry at the ITIES has been used to investigate the transfer characteristics of charged drug molecules, for example, the Galvani potential difference ($\Delta_o^w\varnothing$) for the ion transfer and the Gibbs energy of transfer, which is directly related [22]. Previous studies by Girault and co-workers [23,24] have shown that the ITIES is a suitable platform for the determination of the partition coefficient of the ionised species, which in turn defines the drug's lipophilicity in biological systems [19,20,22].

Direct drug detection in physiological matrices, such as blood and blood-derived samples, is important because it offers information regarding circulating levels. Yet, this can be hindered due to drug–protein binding [25]. The drug–protein interaction in blood plays an important role in determining drug transportation, absorption, distribution, metabolism and excretion [26–28]. Serum albumins are present at the highest abundance in blood (ca. 60% of the total albumin) [29,30], and these proteins exhibit high affinity towards drugs [26–28,31]. In addition, the pharmacological activity of drugs relates to their free concentration in blood [25]. The measurement of drug–protein interactions has seen the emergence of a number of novel label-free strategies [32]. In this study, albumin from bovine serum (BSA) is employed due to the fact that human and bovine serum albumins are homologous proteins [29,33,34]. BSA is a highly water-soluble globular protein, which has a molar mass of 69,000 amu and a hydrodynamic radius of ca. 3.25 nm [25,35]. BSA also has a low isoelectric point (pI of 5.4) and high negative net charge at neutral pH [36].

In this report, emphasis is placed on the electrochemical behaviour of protonated ractopamine ($RacH^+$) at the micro-ITIES array. Quantitative methods that involve the detection of $RacH^+$ by simple ion transfer at the water | 1,6-dichlorohexane (DCH) micro-interface array are presented, using CV and linear sweep stripping voltammetry (LSSV). Stripping analysis at micro-liquid | liquid interface arrays is appropriate for analyte detection in media such as biological fluids, soil extracts and water [37], thus this technique is examined in this study. The thermodynamic parameters for the transfer of ionisable ractopamine are discussed. In addition to the analytical parameters, the influence of the interfering substances, including serum protein, towards $RacH^+$ detection are also reported.

2. Materials and methods

2.1. Reagents

All reagents used were purchased from Sigma-Aldrich Pty. Ltd., Australia and used without further purification, unless stated otherwise. D-Glucose and sodium chloride (NaCl) were purchased from Ajax Finechem Pty. Ltd., Australia, L-ascorbic acid and potassium phosphate monobasic (KH_2PO_4) from BDH Laboratory Supplies, Australia, and sodium sulfate (Na_2SO_4) from Chem-Supply Pty. Ltd, Australia.

The aqueous phase solution of 10 mM lithium chloride (LiCl) was prepared in ultrapure water (resistivity of 18 MΩ cm) from a Milli-Q water purification system (Millipore Pty. Ltd., North Ryde, NSW, Australia). The organic electrolyte salt was prepared by metathesis of bis(triphenylphosphoranylidene)ammonium chloride (BTPPACl) and potassium tetrakis(4-chlorophenyl)borate (KTPBCl). The organic phase was mechanically stabilized as a polymer gel [38–41] and was composed of the supporting electrolyte (10 mM BTPPATPBCl) and low molecular weight poly(vinylchloride) (PVC) (10% w/v), dissolved in 1,6-dichlorohexane. Prior to the experiments, both the aqueous and organic phase solvents were mutually pre-saturated. The organic reference solution consisted of 10 mM BTPPACl dissolved in 10 mM LiCl (aqueous). Ractopamine hydrochloride and tetrapropylammonium chloride (TPrACl) served as the drug and model analyte species studied, respectively. A stock solution of TPrACl was prepared in 10 mM LiCl while the stock solution of ractopamine hydrochloride was prepared in methanol (MeOH) due to its low solubility in water [15].

In the interfering substances study, the interferents were prepared in 1 mM PBS solution as the supporting electrolyte, which contained 1 mM phosphate buffer, 0.27 mM potassium chloride (KCl) and 13.7 mM NaCl. D-Glucose, L-ascorbic acid, KCl, NaCl, Na_2SO_4, glycine and urea were the interfering substances studied, and the